

EXHIBIT 4

**OC7.5**

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A novel filamin A-binding molecule may significantly enhance SST2 antitumoral actions in GH-secreting PitNET cells

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The main target of pharmacological therapy for growth hormone (GH)-secreting pituitary tumors (GH-PitNET) is the somatostatin receptor type 2 (SST2). However, approximately half of patients treated with octreotide, an SST2 agonist, show a low response rate or are octreotide-resistant. Here we present mechanistic data that shows co-treatment with simufilam, a novel oral therapeutic candidate, enhances sensitivity to octreotide. We previously showed that the cytoskeleton protein filamin A (FLNA) is recruited to bind SST2 upon agonist stimulation, and this interaction is required for SST2 signaling in GH-PitNET cells. However, when phosphorylated at Ser2152, FLNA no longer enables SST2 signaling and all SST2 anti-tumor effects are abolished. Simufilam is a FLNA-binding small molecule shown to modulate FLNA's conformation and its interactions with partner proteins in disease states. We postulated that simufilam may restore FLNA's linkage to SST2 and therefore FLNA's ability to enable SST2 signal transduction. To test this hypothesis, we assessed simufilam's effects on FLNA phosphorylation, FLNA-SST2 complex formation, SST2 signal transduction, GH secretion and cell proliferation/apoptosis, in human primary cultured GH-PitNET cells and in

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the rat pituitary tumor cell line GH4C1. Simuflam treatment reduced FLNA phosphorylation on Ser2152 in GH4C1 cells ($-28\pm13\%$ after 10 min, $P<0.01$ vs basal) and in primary human GH-PitNET cells (-59%). Additionally, FLNA-SST2 complexes in GH4C1 cells fell below basal levels after 1h octreotide treatment ($-29\pm6.8\%$, $P<0.05$ vs bas) but were still elevated after 1h co-incubation with octreotide+simuflam ($135\pm19.7\%$, $P<0.05$ vs bas). Simuflam did not affect the ability of octreotide to inhibit GH secretion in GH4C1 or primary GH-PitNET cells that are *in vitro* responsive to octreotide; however, a combination of simuflam+octreotide reduced GH secretion in primary GH-PitNET cells that are *in vitro* resistant to octreotide ($n=2$) ($-42\pm3.5\%$, $P<0.001$ vs bas). Simuflam slightly reduced cell proliferation ($-15\pm10.1\%$, $P<0.05$ vs bas) and ERK phosphorylation ($-21\pm18.8\%$, $P<0.05$ vs bas), while increasing cell apoptosis ($+17.8\pm7.3\%$, $P<0.05$ vs bas) in the GH4C1 cell line. Interestingly, co-treatment with simuflam+octreotide in GH4C1 potentiated the pro-apoptotic effect of the single drugs ($+13\pm5\%$ octreotide, $P<0.001$ vs bas; $+36.8\pm9.2\%$ octreotide+simuflam, $P<0.01$ vs bas, $P<0.05$ vs octreotide or simuflam alone). In conclusion, simuflam reduced FLNA phosphorylation, enhanced and prolonged the octreotide-induced FLNA-SST2 interaction and promoted SST2 signal transduction in human primary cultured GH-PitNET cells. These data suggest that co-treatment with simuflam may enhance the efficacy of octreotide or other somatostatin analog drugs in the management of pituitary tumors.

My recently viewed abstracts


[A novel filamin A-binding molecule may significantly enhance SST2 antitumoral actions in GH-secreting PitNET cells \(<1 min ago\)](#)


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A novel filamin A-binding molecule may significantly enhance SST2 antitumoral actions in GH-secreting PitNET cells

Erika Peverelli

Milan, Italy





European Society
of Endocrinology

CONFLICT OF INTEREST

Erika Peverelli

☐ I have the following potential conflicts of interest to report:

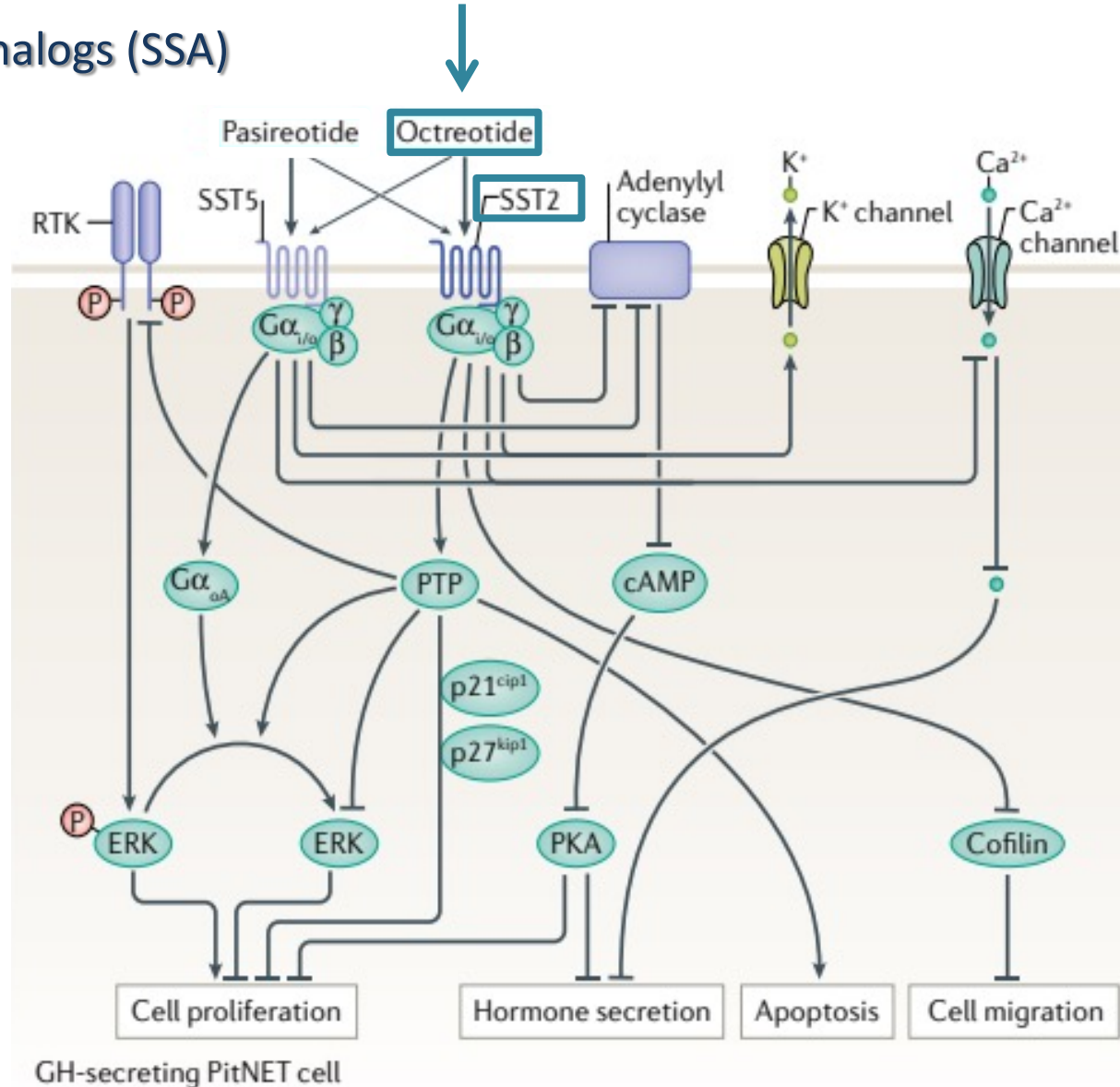
- ☐ Research Contracts
- ☐ Consulting
- ☐ Employment in the Industry
- ☐ Stockholder of a healthcare company
- ☐ Owner of a healthcare company
- ☐ Other(s)

X I declare that I have no potential conflict of interest.

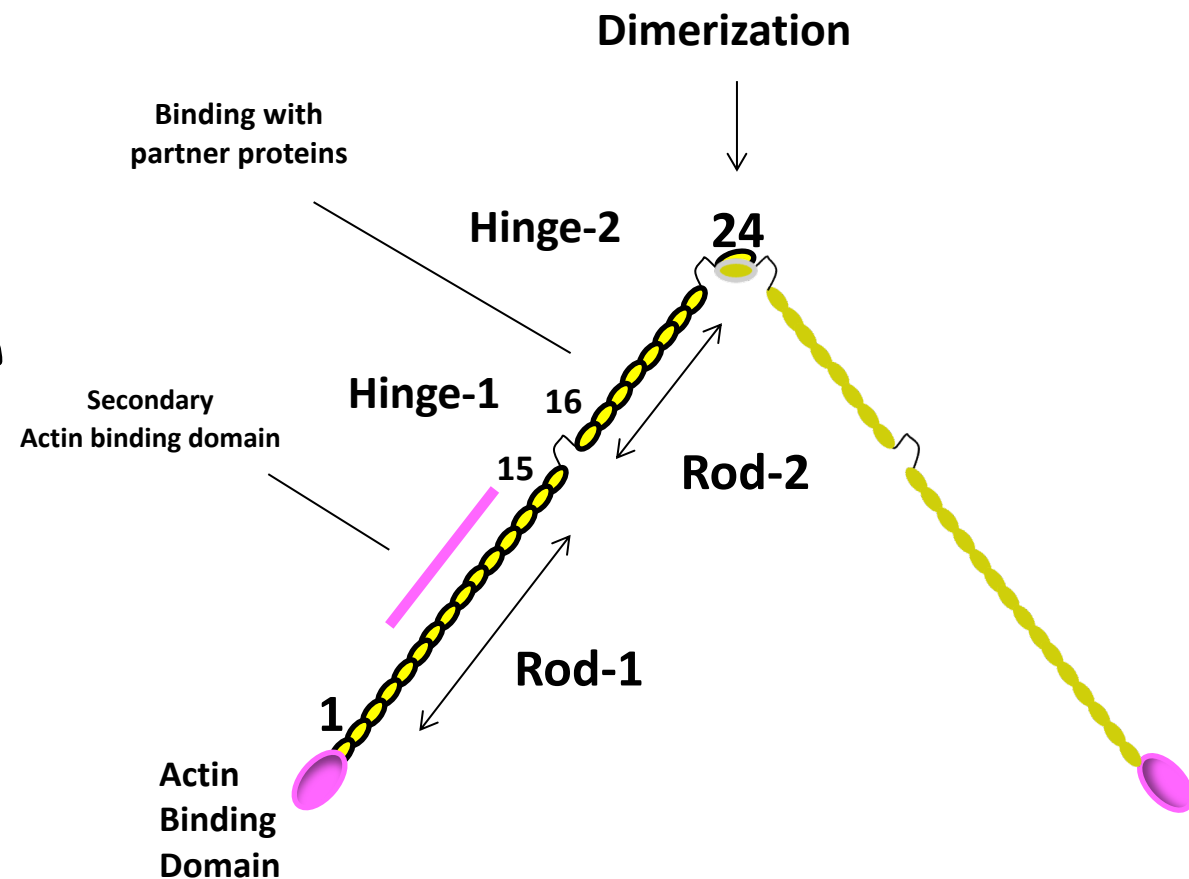
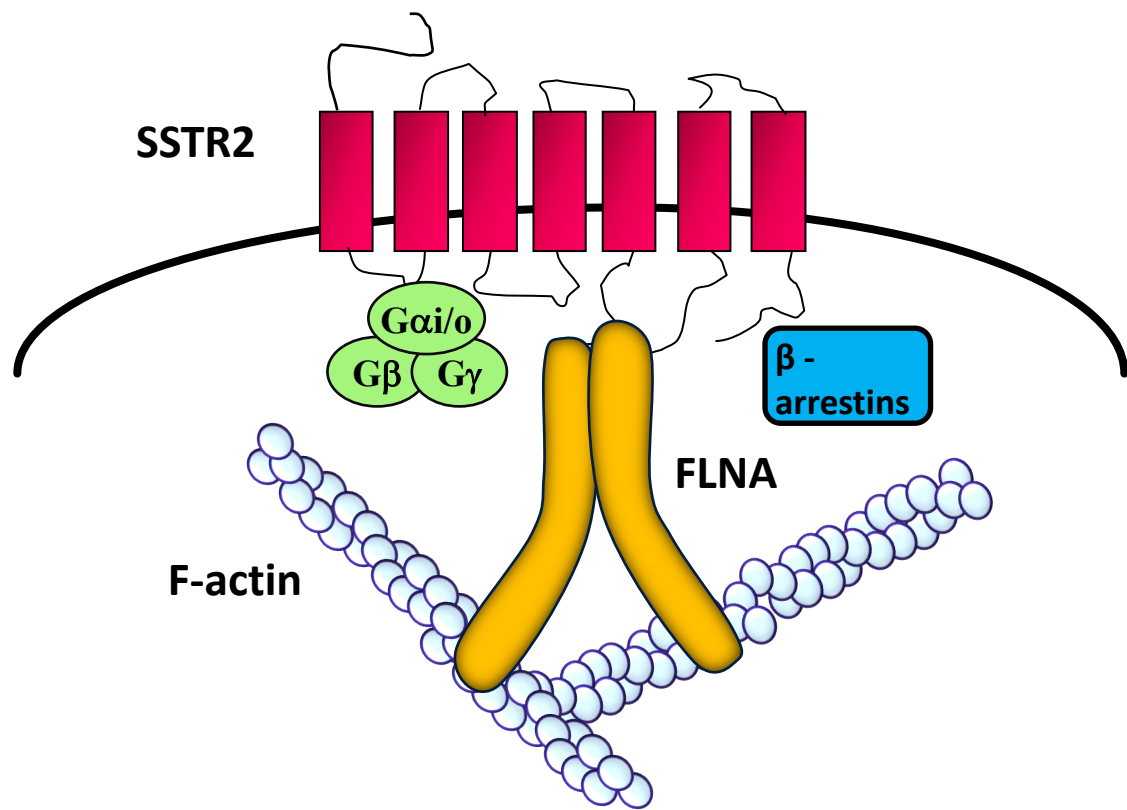


Simufilam is a proprietary compound of
Cassava Sciences provided to E.P. under a
Material Transfer Agreement

Somatostatin analogs (SSA)



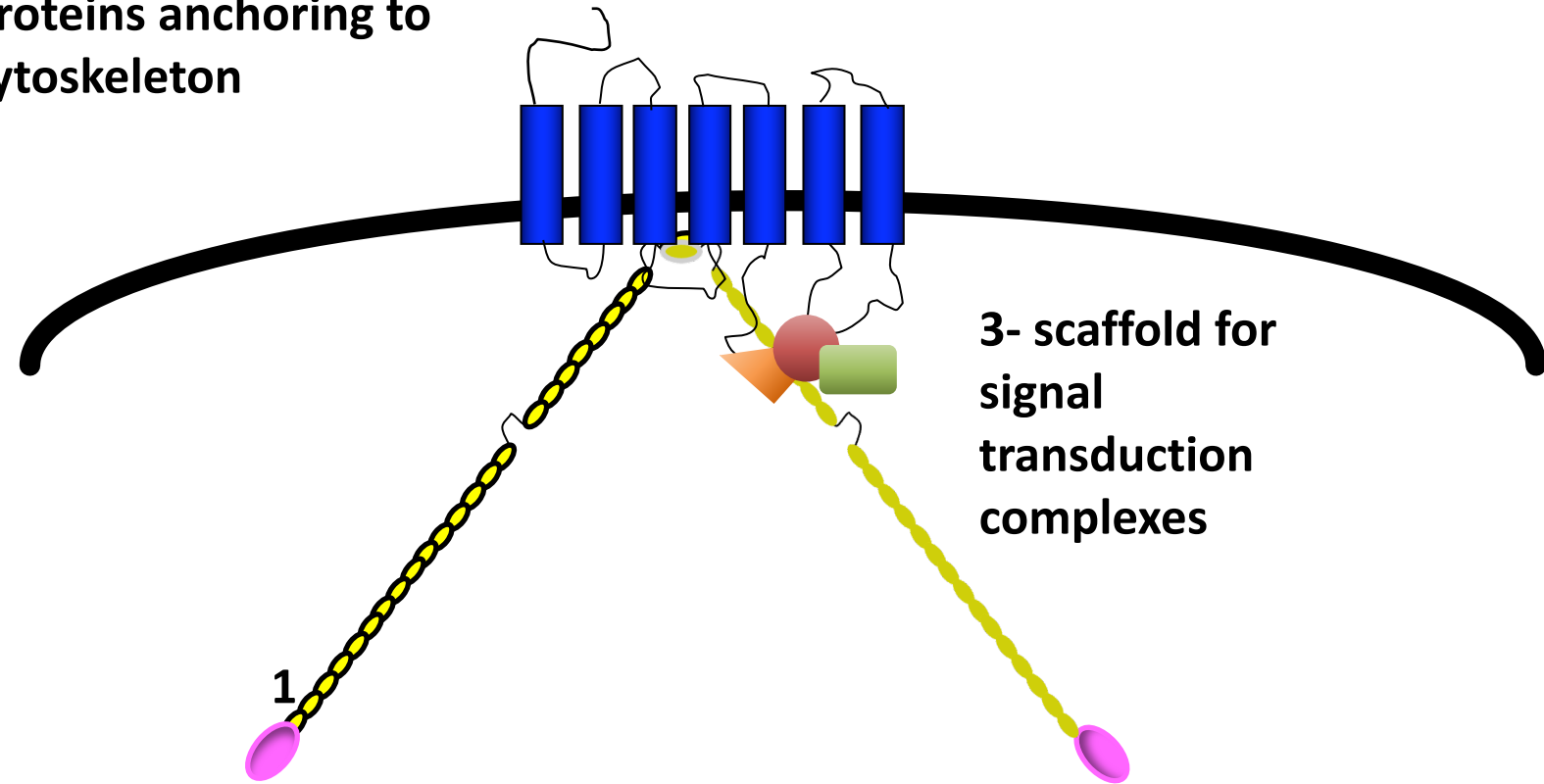
- ❖ Inhibition of hormone secretion
- ❖ Inhibition of cell proliferation



FLNA

- STRUCTURAL ROLE:
- FUNCTIONAL ROLE:

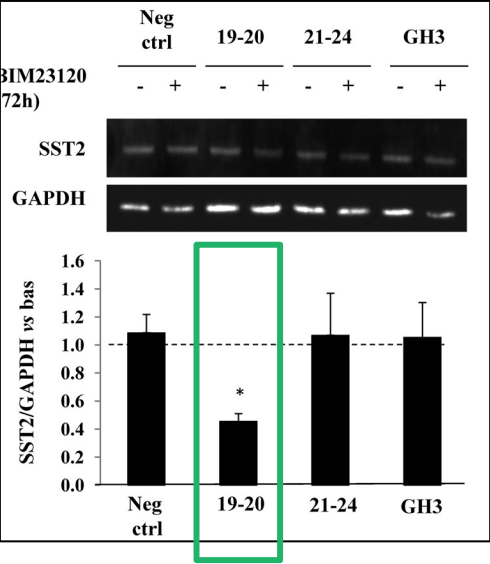
2- transmembrane
proteins anchoring to
cytoskeleton



3- scaffold for
signal
transduction
complexes

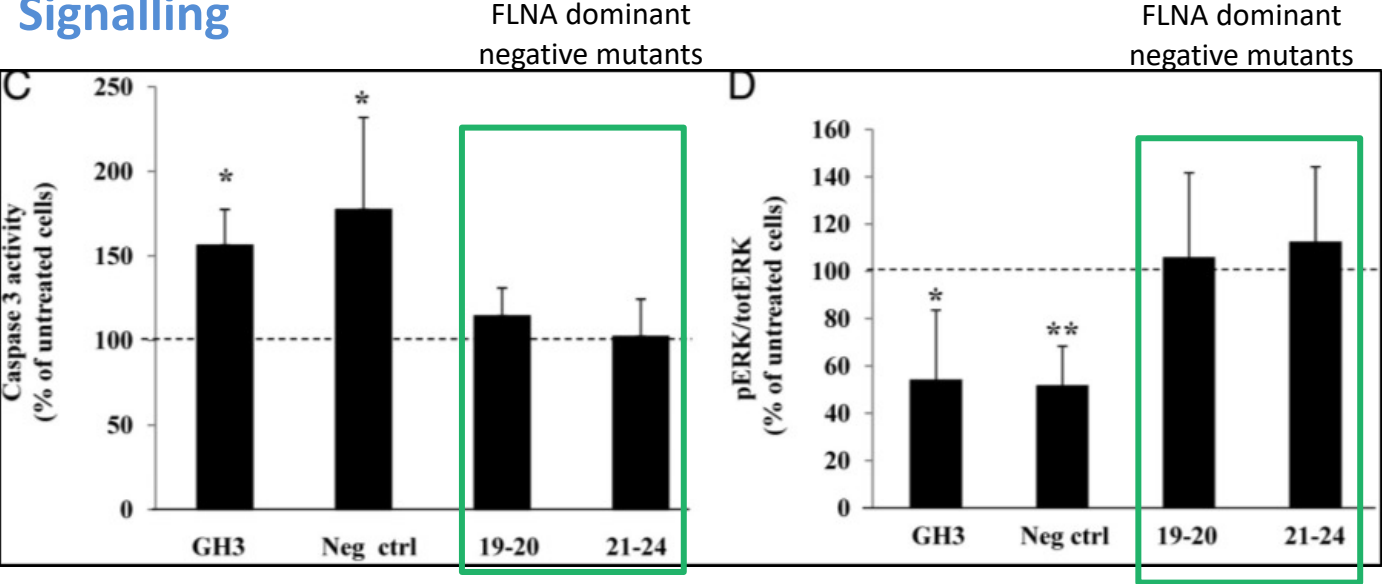
1-Actin filaments crosslinking

Stability



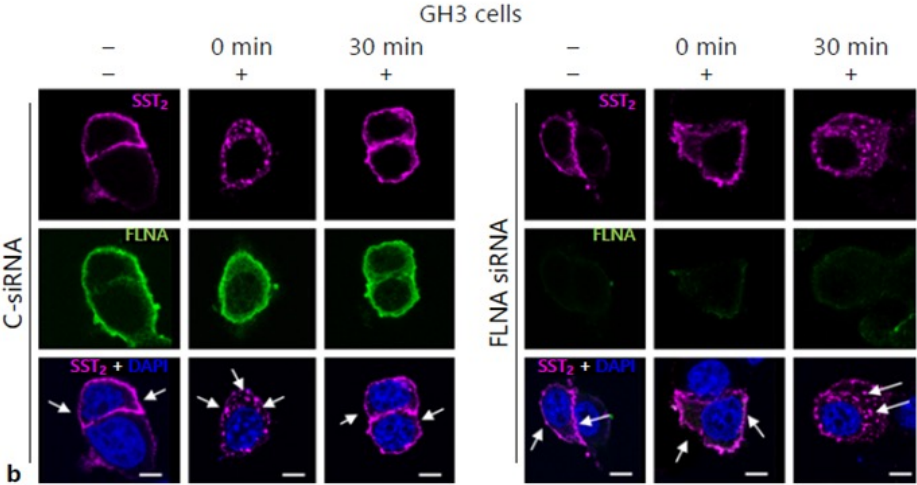
Peverelli E, et al. Endocrinology 2014

Signalling

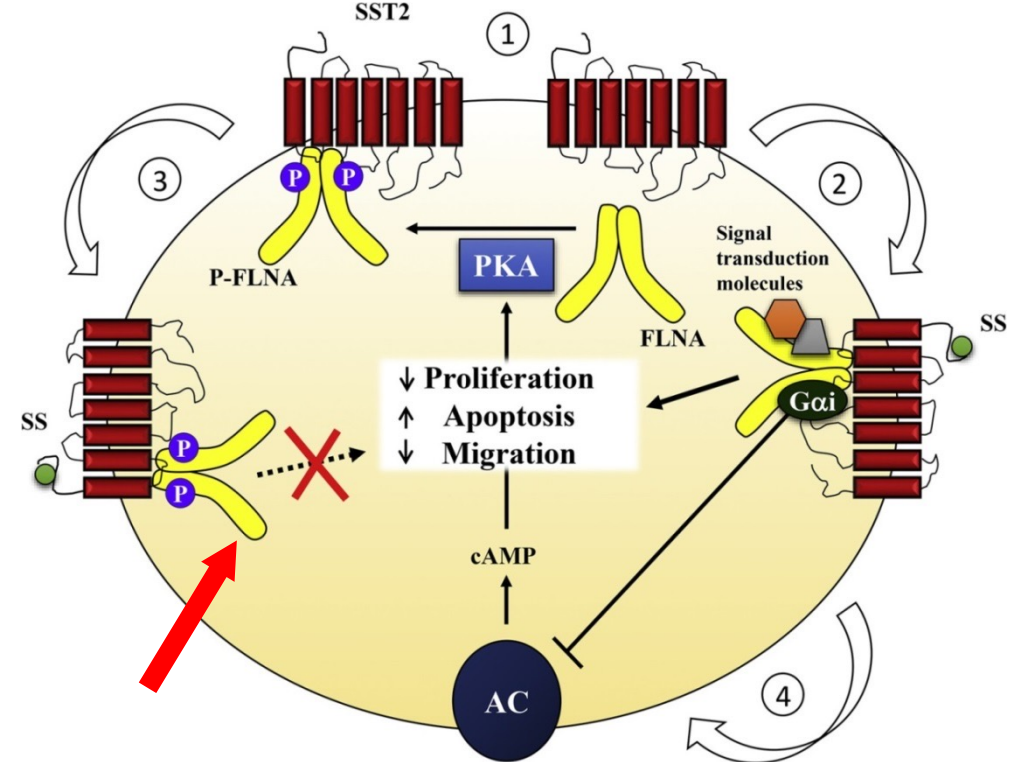
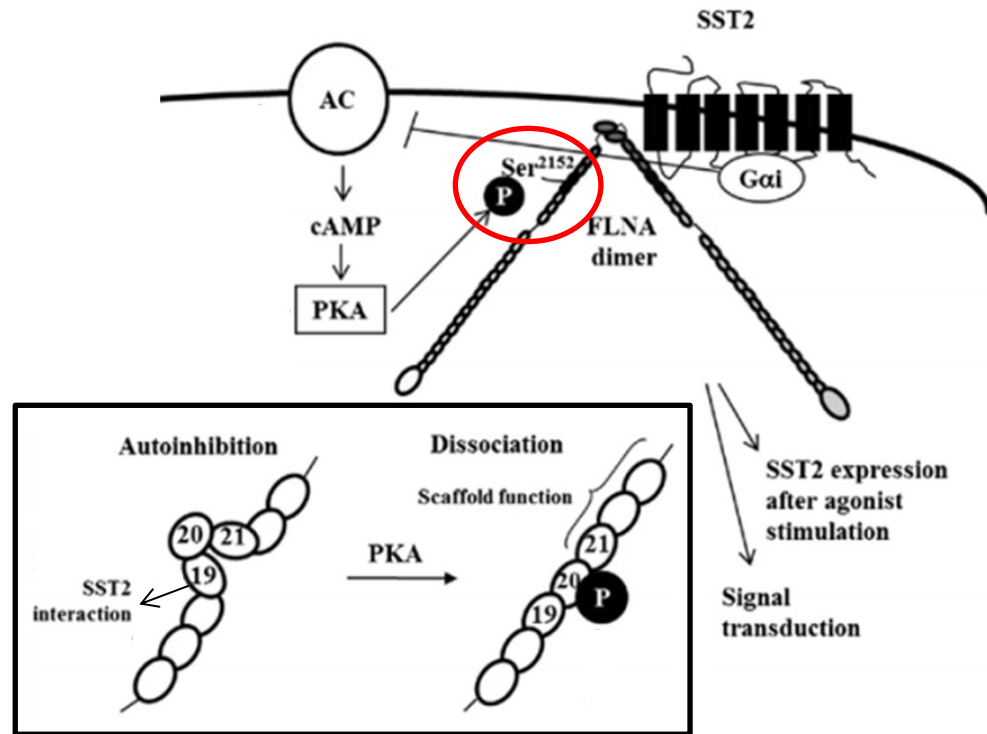


Peverelli E, et al. Endocrinology 2014

Internalization and recycling

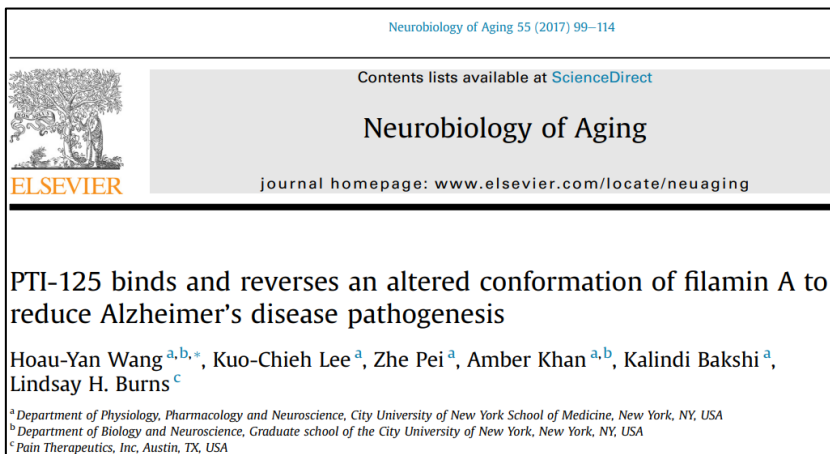


Treppiedi D, et al. Neuroendocrinology. 2020



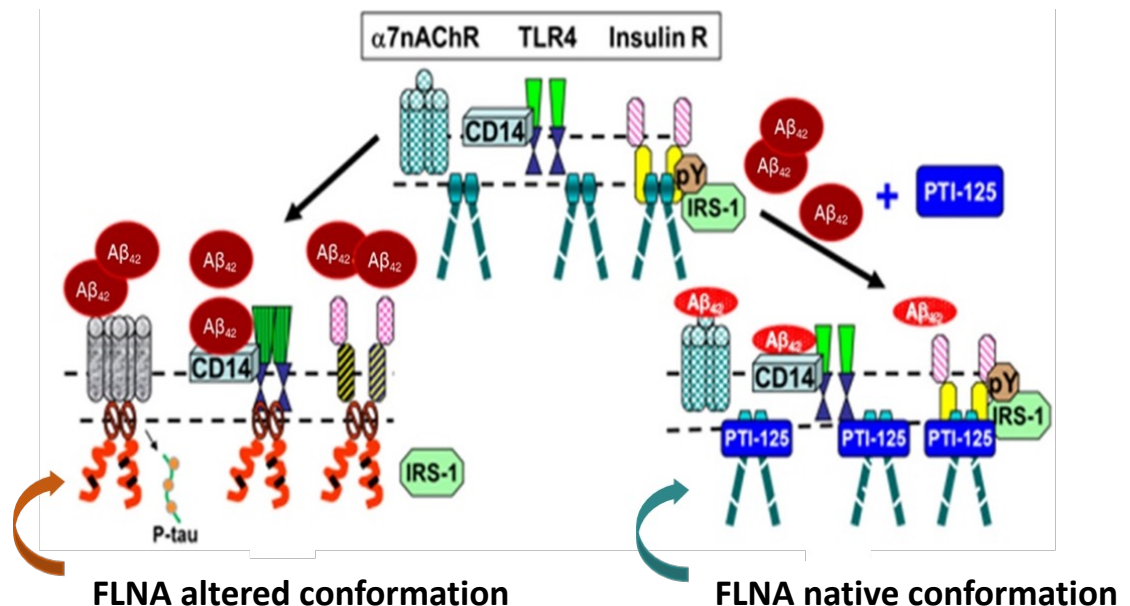
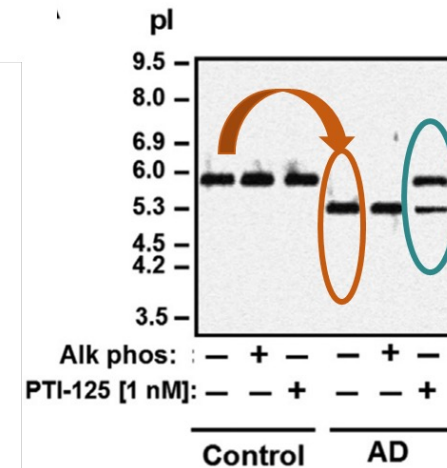
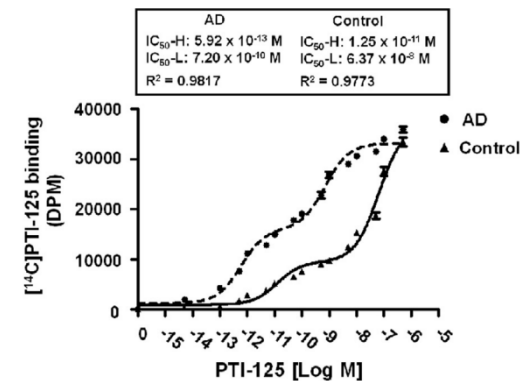
Peverelli et. al/ Cancer Lett (2018)

**P-FLNA modulation:
new pharmacological strategies
to overcome SSA resistance?**



Simufilam:

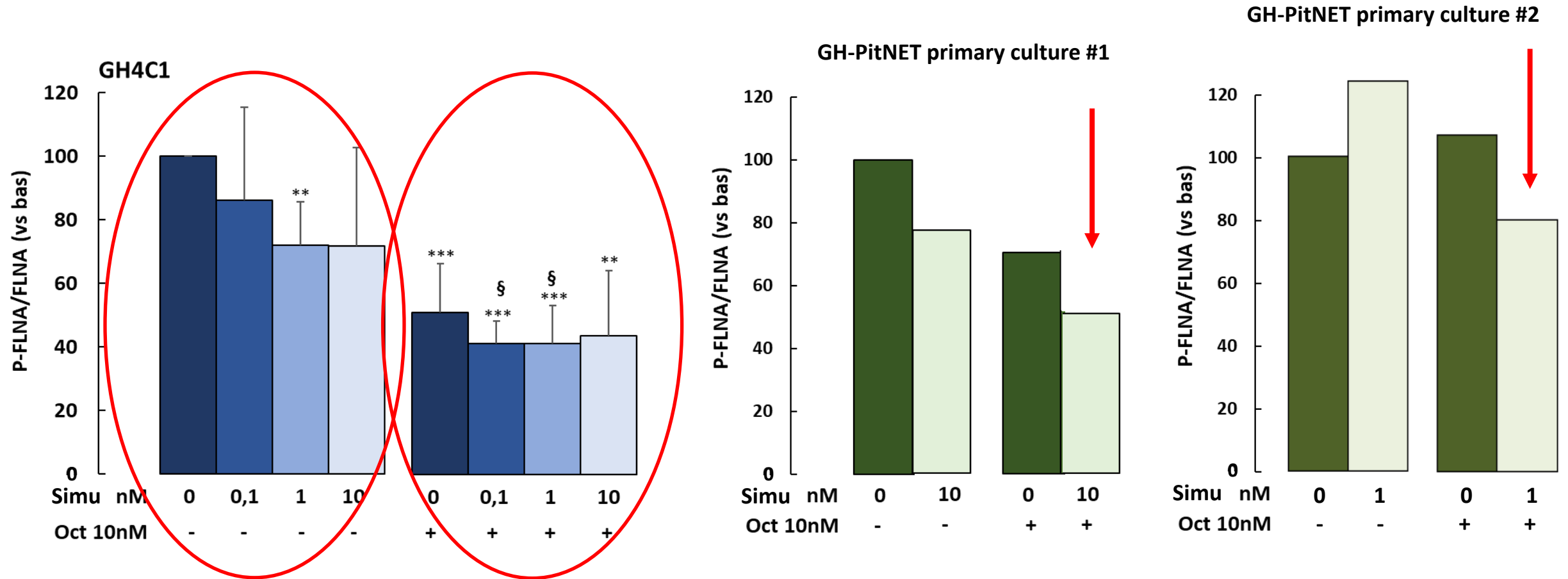
- binds with higher affinity an altered conformation of FLNA
- restore the FLNA native conformation
- change the affinity of FLNA for partner proteins



Aim

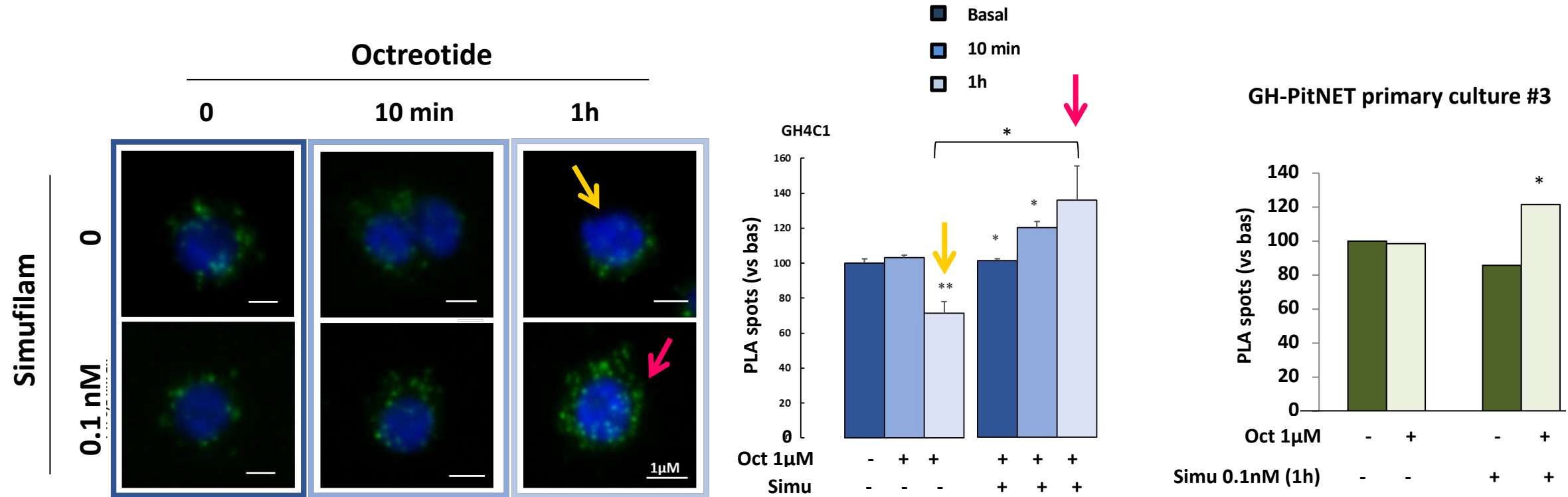
To test Simufilam effects on FLNA phosphorylation, FLNA-SST2 binding, and SST2 signal transduction

- Rat GH-secreting tumoral pituitary cell line GH4C1
- Primary cultured cells derived from surgically removed human GH-secreting tumors



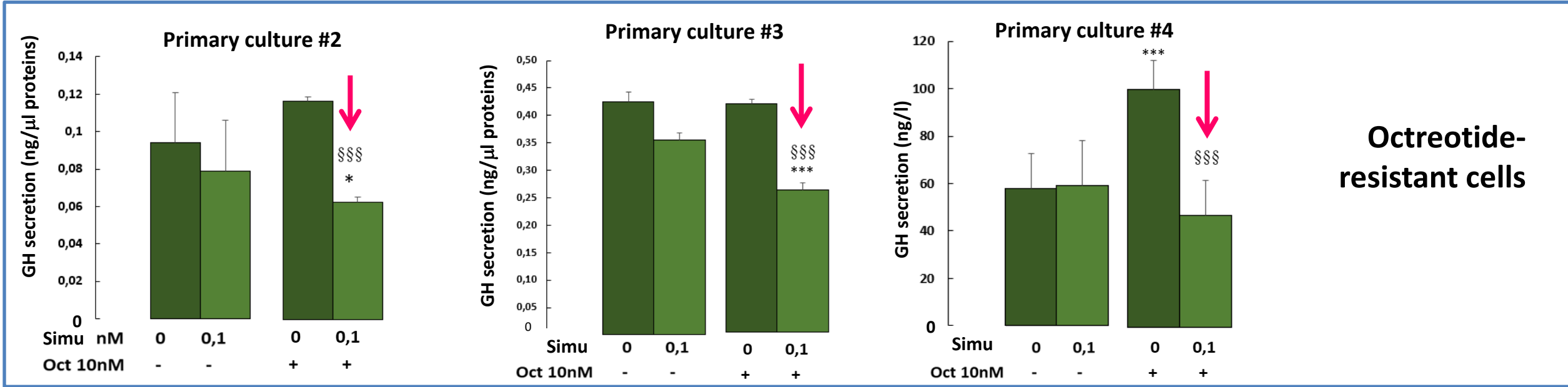
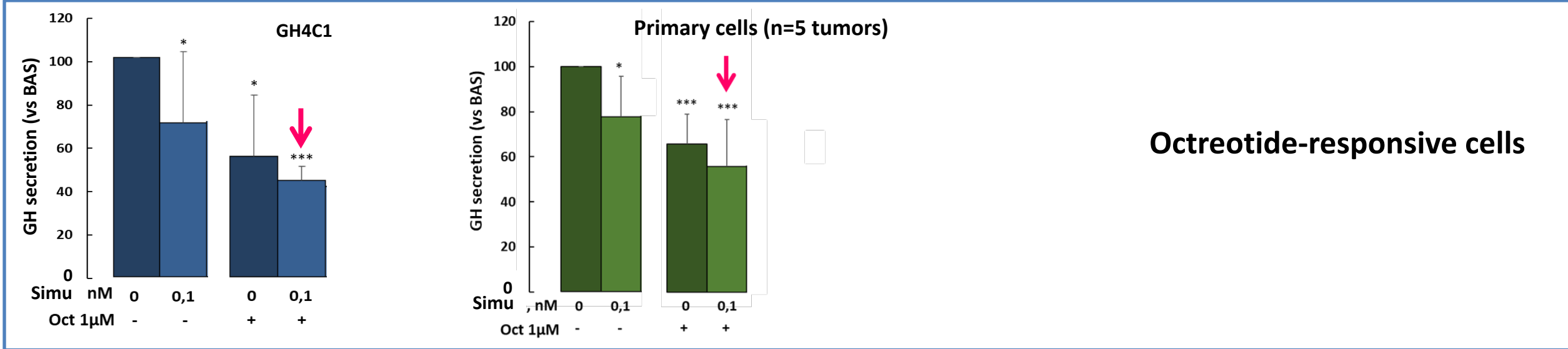
Simufilam and octreotide co-treatment increased FLNA/SST2 interaction

Proximity Ligation Assay (PLA)

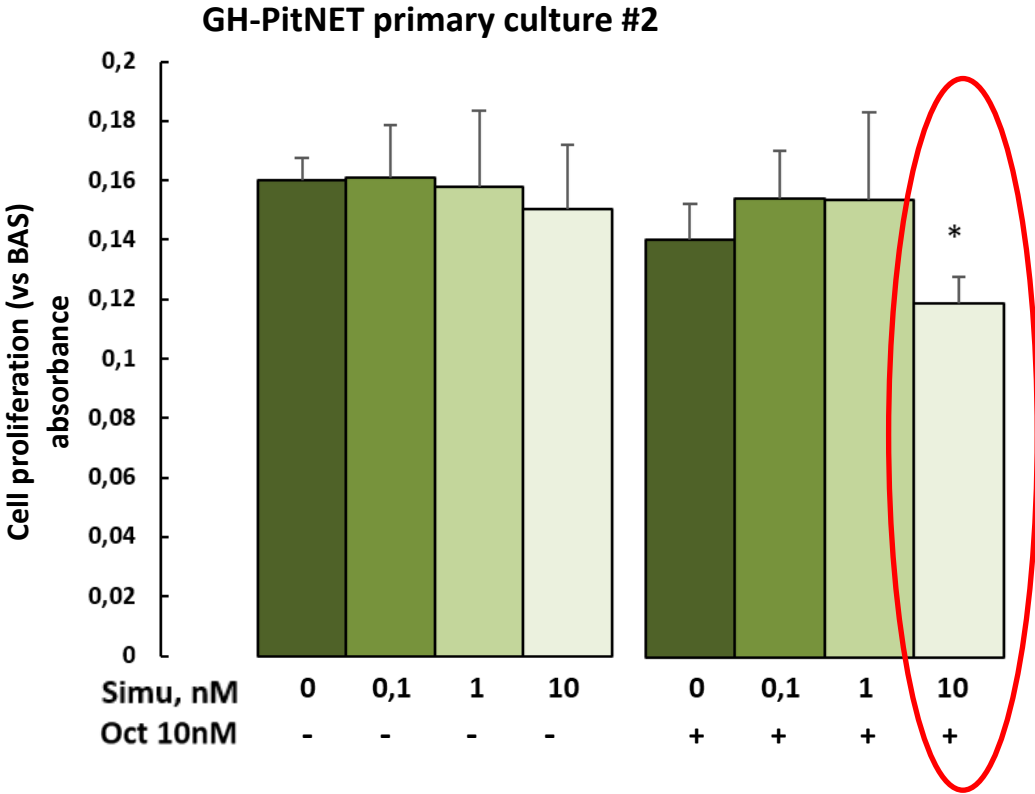
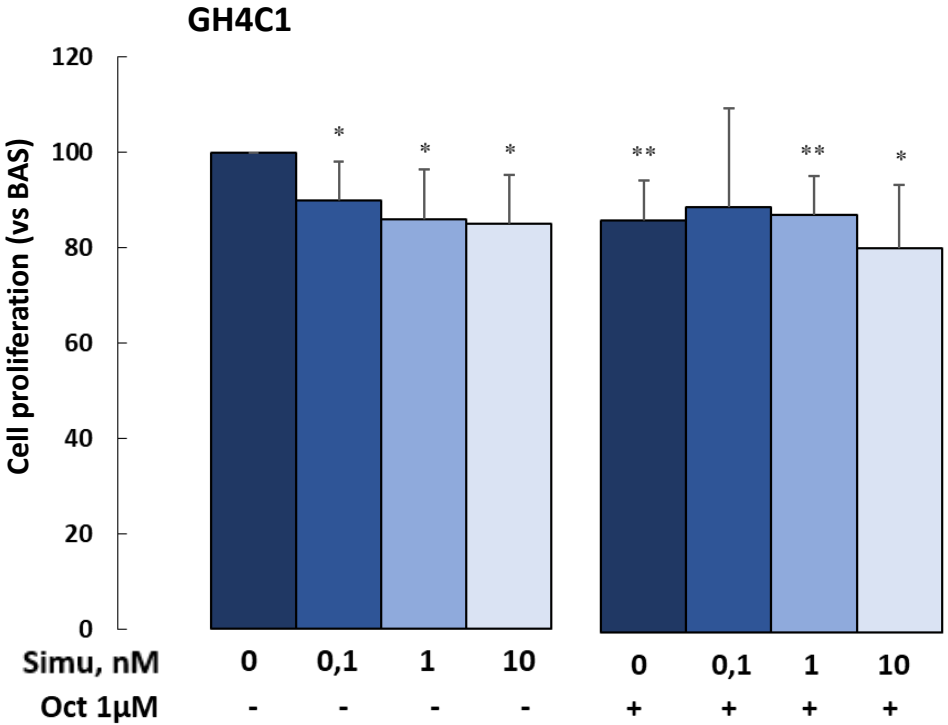


Case 1:22-cv-09409-GHW-OTW Document 121-4 Filed 04/29/24 Page 17 of 21

Simufilam and octreotide co-treatment reduced GH secretion in octreotide-resistant tumor cells

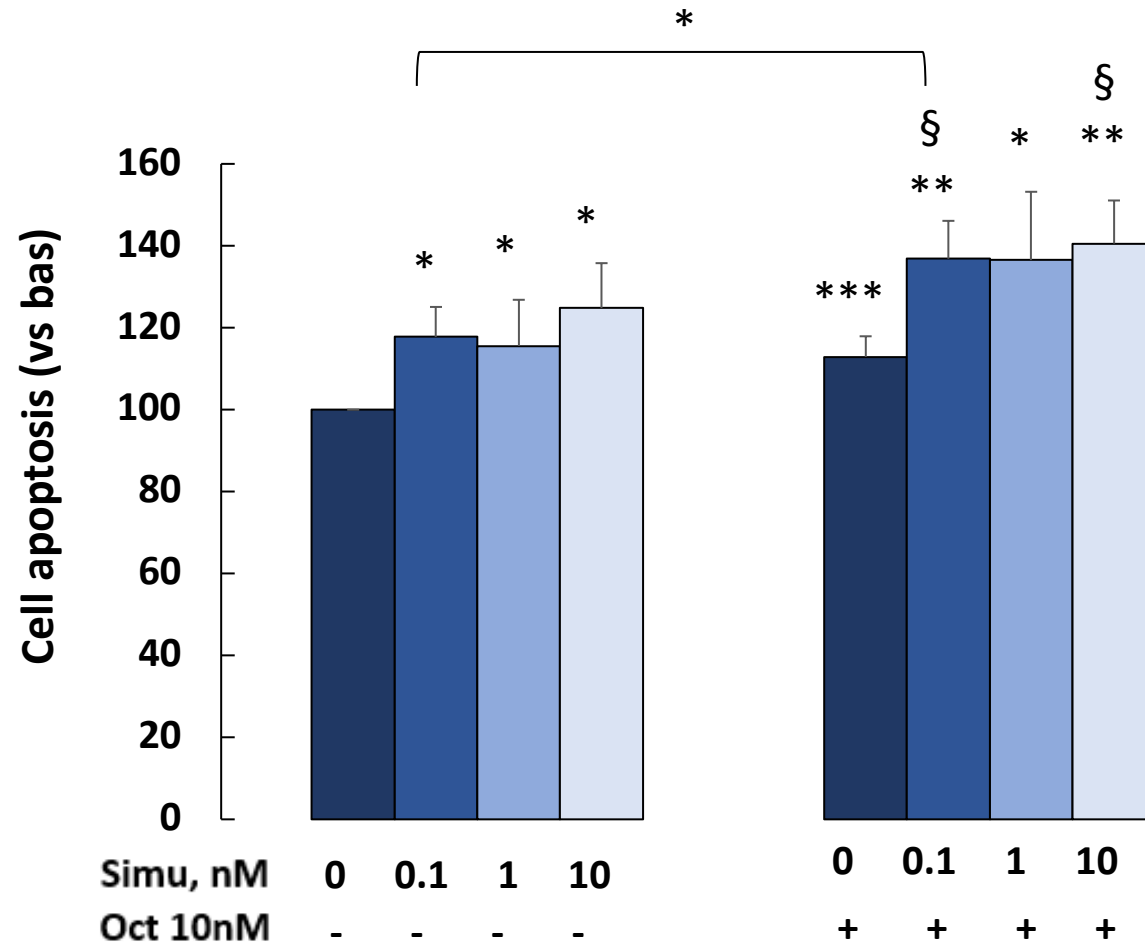


Simufilam and octreotide co-treatment reduced cell proliferation in octreotide-resistant tumor cells



*,p<0.05 vs bas; **, p<0.01 vs bas

Simufilam potentiated the pro-apoptotic effect of octreotide in GH4C1 cells



*, p<0.05 vs bas; **, p<0.01 vs bas; ***, p<0.001 vs bas; §, p<0.05 vs oct;

Conclusions

In GH-secreting PitNET cells, simufilam:

- **reduced FLNA phosphorylation**
- **enhanced and prolonged FLNA-SST2 interaction**
- **promoted SST2 signal transduction**
- **restore sensitivity to octreotide in octreotide-resistant tumor cells**



...a combination therapy of Simufilam and SSA may be useful for GH-secreting PitNET resistant to SSA treatment



Thank you for your attention



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